REMARKS

I. Overview

Applicants have reviewed and considered the Office Action dated October 21, 2004 and the references cited therewith. Applicants note that claims 1-27, 32-39 are pending in the instant application. Claims 1, 3-4, 7, 12-14, 20, 22-23, 27, 32-33, 36 and 38 have been amended and support for these amendments can be found in the specification at pages 2, 12, 15, and 35.

The specification has been amended to claim priority to U. S. Patent Application No. 09/229,212. The amendments to the claims are fully supported by the specification as originally filed, and no new matter has been added. Applicants respectfully request reconsideration of the above-identified application in view of the amendments above and the remarks that follow.

II. 35 U.S.C. § 120 - Priority

The first line of the specification has been amended to claim priority to U. S. Patent Application No. 09/229,212 now U. S. Patent No. 6,309,830 B1.

III. 35 U.S.C. § 112, First Paragraph

A. Claims 32-39 and Claims 8-13 and 22-27

Claims 32-39 stand rejected under 35 U.S.C. § 112, first paragraph, for lacking enablement over the claimed invention for reasons set forth in the Office Actions mailed 11-21-2003 and 3-10-2004. Claims 8-13 and 22-27 Stand Rejected Under 35 U.S.C. § 112, first paragraph, because the Examiner states that the specification while being enabling for practicing the claimed invention *in vitro*, does not reasonably provide enablement for practicing the full scope of the claimed invention *in vivo*. These rejections are traversed and will be addressed collectively.

The Examiner states that the Applicants have only provided *in vitro* data using Xeroderma pigmentosum group A cells demonstrating the ability of a human Arg opal suppressor tRNA to partially restore the activity of the nonsense mutated XPAC gene.

The Examiner cites the Wands factors stating that when these factors are weighed the Examiner concludes that undue experimentation would be required to practice the invention through the full scope of the claims, and therefore the invention is not enabled. Because the amount of experimentation necessary to determine the appropriate modes of delivery of the suppressor tRNA gene sequence into an animal to assess the possible toxicological effects the treatment may have on the translation of other known and unknown proteins in each cell and said animal is beyond the scope of one of ordinary skill in the art.

Claim 32 has been amended so that it now recites "A method of changing translational products in a cell comprising: introducing to said cell a suppressor tRNA sequence, said tRNA sequence comprising: a human tRNA sequence with a total length of less than 150 nucleotides comprising no more than twenty 3' flanking residues and no 5' flanking residues, said sequence encoding an anticodon region for pairing with mRNA; an anticodon sequence contained within said anticodon region which has been modified to recognize and pair with a codon different from that which is originally recognized. This amendment is supported in the Specification at page 13.

The Examiner states that the Specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with the claims, for the reasons set forth in the rejection of claims 32 and 37 under 35 U.S.C. § 112, first paragraph. The Examiner states that the adequate delivery to desired target cells *in vivo*, and the subsequent adequate expression of recombinant constructs such as recombinant human tRNA structural genes, are highly unpredictable and results obtained *in vitro*

cannot be extrapolated to situations *in vivo*. The Examiner cites several articles on the technology of codon context to support this position. Applicants respectfully traverse the grounds for these rejections.

Applicants disagree with the Examiner's suggestion that the cited references support the position that the application of tRNA suppressors are highly unpredictable and would not be effective suppressors because Atkinson et al teach the effectiveness of suppressors differ both with respect to which codons are being read and on the contexts in which termination signals lie. (Nucleic Acids Res. 22(8): 13-27-1334, at p. 1332; Beir et al, § Codon Context Effects).

However when one reads the articles as a whole, as required under MPEP 2141.02, the references actually teach the potential usefulness of tRNA suppressors in cells, and more importantly fail to provide any reasonable basis to doubt the ability of the tRNA suppressors to read a nonsense codon and insert an amino acid in a nascent polypeptide in cells as observed in the instant invention.

The paper by Atkinson is a review of nonsense mutations and natural stop codons. The statements alluded to by the Examiner concerning extrapolations from *in vitro* tRNA suppression of a nonsense codon to predictions about *in vivo* efficiency and toxicity is only one part of the paper. When read in its entirety the author is merely stating a well known fact that 3' codon context plays a role in the efficiency of human nonsense suppressors. The Examiner described that Li et al cite that one major concern for the use of tRNA suppressors in human gene therapy is their potential toxic effects. The Examiner states that the introduction of a tRNA suppressor may potentially cause a read through in other genes, subsequently leading to changes in the function of their gene products. The Examiner states that this observation of Li et al suggests that the effects of a tRNA suppressor in a cell, as it relates to non-specific gene regulation, is

unpredictable. Atkinson addresses these concerns in her paper when she states that "in the case of gene therapy by a suppressor tRNA the level of tRNA could be adjusted so that the readthrough by at a natural stop codon may be as little as 5-10% ... Readthrough of this intensity at natural termination codons, may not present so drastic an outcome in the presence of 90-95% of correctly terminated polypeptide chains." Atkinson, at page 1333, 1st column, 2nd paragraph. Therefore, even if some readthrough occurs by a tRNA suppressor, it may not be sufficient to alter phenotype or cause toxicity. Therefore, the statements by the author do not demonstrate the unpredictability of tRNA suppressors *in vivo*, rather it merely states the obvious that the 3' codon context plays a role in suppression of nonsense mutations. The teaching of the paper by Atkinson and the other cited section by Beier provide no evidence that extrapolation from *in vitro* data on tRNA suppressors to *in vivo* data is unpredictable.

Beier reviews "codon context effects, i.e. primary sequences and secondary structures in the vicinity of 'leaky' stop codons that influence the efficiency of suppression." Beier, at page 4767, column 2, 1st paragraph. When read in its entirety the author is merely stating a well known fact that 3' codon context plays a role in the efficiency of human nonsense suppressors. Nowhere in the Beier reference does the author suggest that results with tRNA suppressors *in vitro* would not be achieved *in vivo*. In fact, statements in the paper by Beier support the fact that tRNA suppressors will work *in vivo*. For example, the author states that "an increasing number of reports have been published recently indicating that natural suppressor tRNAs have apparently corrected deleterious nonsense mutations within open reading frames from human ... genome *in vivo*." Beier, at page 4780, column 1, 1st paragraph. Therefore, the paper Beier and the paper by Atkinson teach that while 3' codon context plays a role in suppression, they do not support the conclusion that the data from *in vitro* studies is not predictive of *in vivo* activity.

Evidence that the tRNA suppressor gene functions as described is found in the Applicants' own work as well as in others skilled in the art. See Beier, at page 4780, column 1, 1st paragraph. Applicants' specification provides sufficient guidance to allow one of skill in the art to practice the claimed invention. The Specification, at page 12, discloses tRNA sequences that are available through GenBank as well as scientific publications, including Sprinzl, Mathias et. Al., Nucleic Acids Research, volume 12, Supplement "compilation of tRNA Sequences" pgs, r1-r57 (1984); Schimmel, P. R., et. Al. Editors, "Transfer-RNA: Structure, Properties, and Recognition, Cold Spring Harbor Labs New York 1979.; Agris, P. F., (1983) "The Modified Nucleosides of Transfer RNA, II, Alan R. Liss Inc., New York (Buckland RA et al., "A cluster of tRNA genes into [DRNI, TRR3, DDRAN] on the short arm of human chromosome 6", Genomics, 35 164-171 (1996)). One skilled in the art would be able to identify tRNA sequences of the present invention. tRNA is typically composed of 76 nucleotides arranged in a cloverleaf secondary structure with four stems and three loops. The loops with their associated hydrogen-bonded stems are designated as the dihydrouridine (D), anticodon, and TΨC-stem loops. The anticodon loop is ably predicted by modeling systems such as MFOLD to approximate the stem-biloop structure of the second domain of tRNA. See Beebe K et al, (2003), *J. Biol. Chem.*, 278(46): 45056-45061, Walter, A. E. et al, (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 9218–9222 (describing the use of the Zuker program to generate RNA secondary structures).

Therefore, tRNA sequences may be obtained from multiple sources, and their potential structures identified by using programs and modeling systems that predict secondary and tertiary structures, such as the anticodon loop. The instant invention uses less than a full length tRNA sequence, specifically the invention uses no more than 150 nucleotides, rather it includes only the structural component of the gene, including around 15 bases from the 3' flanking region and none

of the 5' noncoding region. One skilled in the art will likely have an advanced degree, and if necessary, would be able to look to state of the art databanks, publications, or modeling systems, to identify the claimed tRNA sequences.

Furthermore, one skilled in the art would then be able to design and make an anticodon nucleotide sequence whose recognition sequence differs from the original tRNA anticodon sequence. Several examples are given at pages 13, and 28-29 where the construction of an arginine opal tRNA suppressor is described. One skilled in the art would have knowledge of the genetic code -- that triplet nucleotides code for an amino acid. One skilled in the art would also understand the relationship between the codon on mRNA and the anticodon on tRNA, that is, that the three base sequence in the anticodon is complementary to the codon sequence in mRNA. Therefore, the underlying mechanism of the present invention, e.g. changing the translation of sequences by altering the tRNA anticodon sequence, is widely applicable since it is not specific to any particular disease state. For example, point mutations in sickle cell anemia and cystic fibrosis genes both create aberrant proteins, but the effects of each mutant manifest in completely different diseases. Therefore, a variety of diseases resulting from missense or point mutations would benefit from the current invention.

Once designed, the oligonucleotide containing the tRNA sequence may be synthesized. Specification, at pages 29. This oligonucleotide can then be cloned using standard molecular techniques into the vector or plasmid of choice. Methods of introducing a tRNA sequence into a cell is described at pages 28-32. In particular Applicants describe transfecting VA13 human fibroblast cells with a herpes simplex virus amplicon vector containing arginine opal suppressor tRNA sequence. Reactivation assays using a reporter gene, CAT, are also described. The use of vectors, transfection techniques, and reporter assays are well known to one skilled in the art.

Based on the foregoing, Applicants respectfully submit that the specification provides enablement commensurate with the scope of claims 8-13, 22-27, and 32-39. The Specification fully enables the claimed invention both *in vitro* and *in vivo* contexts, and the scope of the protection of the claims should properly reflect the claim. It is submitted that the requirements of 35 U.S.C. § 112, first paragraph have been met. Therefore, reconsideration and withdrawal of those rejections are requested.

B. Claims 1-7, 12-23 and 27

Claims 1-7, 12-21, 23, 22 and 27 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Claims 3, 4 and 23 are objected to for the following reasons:

In claim 3, line 1, "an" following "encodes" should be replaced with --a--.

In claim 23, line 2, "an" should be replaced with --a--. Claims 3 and 23 have been amended so that they now recite "a nonsense mutation".

The Examiner cites that claim 1 recites the phrase "[A]n oligonucleotide sequence . . .," the actual patentable subject matter in this case is unclear, since one cannot separate an oligonucleotide from its nucleotide sequence. The Examiner states that therefore it is unclear if Applicants were intending to claim merely the sequence of the oligonucleotide, or an oligonucleotide comprising the recited sequence. The Examiner states additionally, claims 1, line 9, recites the phrase "said oligonucleotide". This phrase is vague and indefinite since it is unclear if the instant claim is drawn to an oligonucleotide sequence or an oligonucleotide having a sequence. Accordingly, Applicants have amended claim 1 so that it now recites "An isolated oligonucleotide". Therefore, claims 2-6 and 21 recite the phrase "The oligonucleotide of claim 1" are no longer indefinite.

Claims 7, 12, and 36 recite "SEQ ID NOS: 1-10 and their complements". The Examiner states that the metes and bounds of the term "complements," as recited in the instant claims is vague and indefinite since it is unclear if the applicants have intended for this term to encompass complements of any particular length or any particular percent complementarity. Applicants amended claims 7, 12 and 36 so that they no longer recite "and their complements" in an earnest effort to advance prosecution and facilitate the allowance of this case.

Claims 13 and 27 recite the "sequence of claim 1". The Examiner states this phrase is vague and indefinite since it is unclear which "sequence" applicants are referring to, the "oligonucleotide sequence," the "human tRNA structural gene sequence," or the "sequence encoding an anticodon region." Accordingly, Applicants have amended claim 13 so that it now recites "the oligonucleotide of claim 1". Claim 27 has been amended so that it now recites " A method for suppressing the effect of nonsense mutations in a nucleotide sequence encoding a protein comprising: introducing to a cell bearing said nonsense mutation a synthetic suppressor tRNA encoded by the oligonucleotide of claim 1."

Claims 14-20 recite the phrase "the nucleotide sequence of claim 1". The Examiner states this phrase is vague and indefinite since it is unclear which "sequence" applicants are referring to, the "oligonucleotide sequence," the "human tRNA structural gene sequence," or the "sequence encoding an anticodon region." Accordingly, Applicants have amended claims 14 and 20 so that they now recite "the oligonucleotide of claim 1".

Claims 22 and 27 recite a method of introducing a site specific mutation to a translated protein by introducing a synthetic suppressor tRNA encoded into a cell. The Examiner states it is unclear how a tRNA molecule can site specifically modify a translated protein. The Examiner states that replacing "translated protein" with -nucleotide sequence encoding a protein- would

potentially be remedial. Accordingly, claims 22 and 27 have been amended so that they now recite "A method for suppressing the effect of nonsense mutations in a nucleotide sequence encoding a protein".

In claim 27, line 3, "said cell" is unclear since neither claim 27 nor claim 1, from which it depends, recite a cell. The Examiner states that by replacing "said" with -a- would be remedial. Accordingly, Applicants have amended claim 27 to recite "a cell".

Claims 38-39 recite "a suppressor tRNA sequence" according to claim 1, there is lack of antecedent basis for this limitation in claim 1. Accordingly, claim 38 has been amended to recite "introducing to said cells an oligonucleotide which encodes a synthetic suppressor tRNA".

In claim 4, line 2, "said" should be replaced with --the--. Claim 4 has been amended so that it now recites "The oligonucleotide of claim 1 wherein said oligonucleotide encodes said synthetic suppressor tRNA in tandem."

In light of the above amendments, claims 1-7, 12-21, 23, 22 and 27 are no longer indefinite. Applicants request that the rejections and objections be withdrawn.

C. Claims 1-6, 8-11, 13-25, 27, 32-35, and 37-39

Claims 1-6, 8-11, 13-25, 27, 32-35, and 37-39 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner states that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The Examiner states that although there are multiple known human tRNA structural gene sequences, Applicants are not in possession of the full scope of a human tRNA structural genes encompassed by the instant claims. The Examiner states that the basis of the instant invention

requires the prior knowledge of the structure of a mutant tRNA gene that is associated with the pathogenesis of a certain disease, wherein said mutant tRNA does not provide the correct protein during translation. The Examiner states that without prior knowledge of the mutant tRNA the skilled artisan would not be able to practice the claimed invention.

Applicants respectfully submit that Examiner misunderstands the invention. The invention is not directed to a mutant tRNA that does not provide the correct protein during translation, nor is it directed to a mutant tRNA gene that is associated with the pathogenesis of a certain disease as Examiner purports. Applicants direct Examiner's attention to the Specification at page 11, first paragraph under Detailed Description, where Applicants provide numerous diseases having point mutations or nonsense mutations that result in various diseases. In nature, no tRNA has an anticodon complementary to stop codons UAG, UAA, or UGA. However, in the present invention, Applicants disclose methods for treating diseases by reversing the effects of mutations by introducing a suppressor tRNA that will recognize a codon with a nonsense mutation (stop codon). The tRNA suppressor with an anticodon region recognizing a nonsense mutation is charged with an amino acid, allowing the incorporation of the correct amino acid into the nascent polypeptide so that the translation is not stopped prematurely. In this way, Applicants believe that the disease with the nonsense mutation may be treated and the effects of the disease may ameliorated.

The Examiner states that if the scope of the human tRNA structural genes recited in the instant claims are not limited to those genes that were previously known in the art as of the filing date of the instant application, further experimentation would be required to fully envision the full scope of the claimed invention. Applicants believe that the Examiner has mischaracterized the invention. As discussed above, the invention is not based on mutant tRNAs that result in

diseases. Furthermore, Applicants' specification provides relevant identifying characteristics of the tRNA suppressor. For example, claim 1 recites "An oligonucleotide sequence which encodes a synthetic suppressor tRNA comprising: a human tRNA structural gene sequence comprising no more than twenty 3' flanking residues and no 5' flanking residues, said sequence encoding an anticodon region for pairing with mRNA; an anticodon sequence contained within said anticodon region which has been modified to recognize a codon different from that which is originally recognized; wherein said oligonucleotide has a total length of less than 150 nucleotides."

The Specification, at page 12, discloses tRNA sequences that are available through GenBank as well as scientific publications, including Sprinzl, Mathias et. Al., Nucleic Acids Research, volume 12, Supplement "compilation of tRNA Sequences" pgs, r1-r57 (1984); Schimmel, P. R., et. Al. Editors, "Transfer-RNA: Structure, Properties, and Recognition, Cold Spring Harbor Labs New York 1979.; Agris, P. F., (1983) "The Modified Nucleosides of Transfer RNA, II, Alan R. Liss Inc., New York (Buckland RA et al., "A cluster of tRNA genes into [DRNI, TRR3, DDRAN] on the short arm of human chromosome 6", Genomics, 35 164-171 (1996)). One skilled in the art would be able to identify tRNA sequences of the present invention. tRNA is typically composed of ~76 nucleotides arranged in a cloverleaf secondary structure with four stems and three loops. The loops with their associated hydrogen-bonded stems are designated as the dihydrouridine (D), anticodon, and TΨC-stem loops. The anticodon loop is ably predicted by modeling systems such as MFOLD to approximate the stem-biloop structure of the second domain of tRNA. See Beebe K et al, (2003), *J. Biol. Chem.*, 278(46): 45056-45061, Walter, A. E. et al, (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 9218–9222 (noting that this reference discloses prior to Applicants' filing date a program to identify and generate RNA secondary structures).

Therefore, tRNA sequences may be obtained from multiple sources, and their potential structures identified by using programs and modeling systems that predict secondary and tertiary structures, such as the anticodon loop. The instant invention uses less than a full length tRNA sequence, specifically the invention uses no more than 150 nucleotides, rather it includes only the structural component of the gene, including around 15 bases from the 3' flanking region and none of the 5' noncoding region. One skilled in the art will likely have an advanced degree, and if necessary, would be able to look to state of the art databanks, publications, or modeling systems, to identify the claimed tRNA sequences.

Furthermore, one skilled in the art would then be able to design and make an anticodon nucleotide sequence whose recognition sequence differs from the original tRNA anticodon sequence. Several examples are given at pages 13, and 28-29 where the construction of an arginine opal tRNA suppressor is described. One skilled in the art would have knowledge of the genetic code -- that triplet nucleotides code for an amino acid. One skilled in the art would also understand the relationship between the codon on mRNA and the anticodon on tRNA, that is, that the three base sequence in the anticodon is complementary to the codon sequence in mRNA. Once designed, the oligonucleotide containing the tRNA sequence may be synthesized. Specification, at pages 29. This oligonucleotide can then be cloned using standard molecular techniques into the vector or plasmid of choice.

Applicants provide relevant identifying characteristics for the tRNA sequences and methods of making tRNA sequences with altered anticodons capable of recognizing nonsense or missense mutations. Based on the foregoing, Applicants respectfully submit that the evidence in the instant application rises to the level necessary to show evidence of possession of the full scope of the claimed invention at the time of the filing. Thus, Applicants respectfully request the

rejections to claims 1-6, 8-11, 13-25, 27, 32-35, and 37-39 under 35 U.S.C. § 112, first paragraph be withdrawn and reconsidered.

IV. Claim Rejections - 35 U.S.C. § 102

Claims 1-3, 5, 8-10, 13-16, 20-24, 27 and 38 stand rejection under 35 U.S.C. § 102(b) as being anticipated by Capone et al.

The Examiner states that since claim 1 and those claims dependent thereon are drawn to an oligonucleotide sequence, and not specifically to an isolated oligonucleotide of a total length of less than 150 nucleotides, the following prior art is applied to the extent that the instant claims are directed merely to a sequence that is disclosed in the prior art.

Claim 1 has been amended so that it now recites "An isolated oligonucleotide" and is not anticipated by Capone et al. Claims 2-3, 5, 8-10, 13-16, 20-24, 27 and 38 dependent on claim 1 are likewise not anticipated by Capone et al. Applicants believe that claims 1-3, 5, 8-10, 13-16, 20-24, 27 and 38 are now in condition for allowance and withdrawal of this rejection is requested.

V. Conclusion

This is a request under the provision of 37 CFR § 1.136(a) to extend the period for filing a response in the above-identified application for one month from October 21, 2004 to February 21, 2005. Applicant is a small entity; therefore, please charge Deposit Account number 26-0084 in the amount of $60.00 for one month to cover the cost of the extension. No additional fees are believed to be due in connection with this amendment; however, consider this a request for any inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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